

## **Selectivity in the Lactational Transfer of PBDEs by Hooded Seals from the Gulf of St Lawrence, Canada**

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### **Introduction**

Transfer of persistent organic pollutants (POPs) to their offspring during lactation has been documented for several marine mammal species including seals (Addison and Brodie 1977; Espeland et al. 1997; Wolkers et al. 2004). However, most of the information currently available is limited to persistent and bioaccumulative organochlorine compounds such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane and metabolites (DDTs). Emerging chemicals such as polybrominated diphenyl ethers (PBDEs) are also occurring in maternal milk but data have almost exclusively been reported from humans (Schecter et al. 2006).

Hooded seals (*Cystophora Cristata*) have the shortest lactation of any marine mammal (4 days). During that short time period, pups gain about 7 kg/day due primarily to the deposition of fat in blubber (Iverson et al. 1995). During that massive transfer of fat, significant transfer of PBDEs from the mother to the pup is expected. Until recently, however, it was not clear if the transfer of PBDEs was proportional to the contamination in the lactating female or if there was selectivity among the PBDE congeners transferred. By comparing the blubber-based body burden of PBDE homolog groups in pups relative to females, Wolkers et al. (2006) concluded that the less brominated PBDE congeners were more efficiently transferred to pups. To the best of our knowledge, there has not been a study comparing specifically PBDE levels and patterns in milk and blubber of the same lactating females at different times during the lactation period.

The objective of this study was to examine the selectivity of the transfer of PBDEs by hooded seals, in the context of their brief lactation period and massive transfer of fat, by measuring PBDEs in biopsied lactating female blubber and maternal milk samples collected twice during the lactation period.

### **Material and Methods**

Tissues samples were collected from 5 seal mother – pup pairs on the pack ice near Magdalene Island in March 1999. All experimental procedures were conducted in accordance with the principles and guidelines of the Canadian Council for Animal Care. The adult females were captured using a slit net, chemically immobilized with a Telezol<sup>®</sup> injection whereas their pups were hand-captured and weighed using 100 kg Salter scale (Table 1). The 5 mother – pup pairs were sampled twice during the lactation period. Blubber and milk samples were collected from the mothers and blubber were collected from their pups. The blubber samples were obtained from the dorsal region of the seals using a biopsy punch (8mm). Milk samples were collected after intramuscular injection of Oxytocin<sup>®</sup>. Samples were stored in polyethylene vials at -20°C until analysis.

Milk and blubber samples were thawed, spiked with  $^{13}\text{C}_{12}$ PCB-170 and chemically dried with anhydrous sodium sulphate before being placed into a soxhlet (milk) or an extraction column (blubber). Lipids and lipophilic compounds in milk and blubber sample were extracted with toluene and dichloromethane (DCM), respectively. Each extraction solution was divided into two fractions. The first fraction was used to determine gravimetrically the lipid content in the tissues. The second fraction received the following  $^{13}\text{C}_{12}$  labeled surrogates BDE-47, -99, and -153 before being reduced in volume for purification. Lipids were removed from the extract by gel permeation chromatography and lipid-free extracts were further cleaned-up by elution through a two-layer column packed with neutral silica and alumina. The hexane fraction was discarded and the DCM-hexane (1:1) fraction was collected, reduced in volume, spiked with an instrument performance solution containing two additional  $^{13}\text{C}_{12}$  PCBs and used for the determination of PBDE congeners. Only PBDE data from milk and blubber samples from lactating females are reported here.

Table 1. Hooded seal mother-pup pairs sampled twice during the lactation period.

Pair	First sampling		Second sampling	
	Postnatal day*	Total weight pup (kg)	Postnatal day**	Total weight pup (kg)
1	2	34	3	43
2	1	25	3	38
3	1	28	3	38
4	1	27	3	unknown
5	1	26	3	unknown

\* Postnatal day was determined from total weight of pup at their first sampling based on the relation calculated from Iverson et al 1995 data (Postnatal day = Pup total weight x 0.156 - 3.257;  $r^2 = 0.99$ ).

\*\* Postnatal day at second sampling was calculated by adding the number of days between the samplings and confirmed with the weight of the pup when available.

PBDE congeners were quantified using a gas chromatograph equipped with an ion trap detector. The ion source was operated in electron impact ionisation mode and the ion trap in mass spectrometry (MS)/MS mode. The chromatography of 39 individual congeners of PBDEs was achieved using a 30 m DB-5MS column with helium as the carrier gas. Concentrations were corrected on the basis of the recovery of the  $^{13}\text{C}_{12}$  labeled surrogates. Limits of quantification for PBDE congener analyses were c.a. 0.1 ng/g lipid weight for blubber and c.a. 0.05 ng/g lipid weight for milk. The precision of analytical methods for blubber samples was assessed by repeated analysis of the whale blubber standard reference material SRM-1945. Only 10 PBDE congeners, namely BDE-28/33, -47, -49, -66, -99, -100, -153, -154, -155, and -183 were systematically detected in SRM-1945. To evaluate the analytical protocol, two cow milk raw samples were spiked with the entire suite of PBDE congeners. The recovery of the 10 PBDEs in cow milk varied from 88% for BDE-66 to 116% for BDE-100. The precision of the method was generally within 30% or slightly higher when levels were lower than 1 ng/g wet weight in the SRM samples. The accuracy of the method was in good agreement (< 20%) with the certified concentrations of the six PBDE congeners reported in SRM-1945 (Wise et al. 2006).

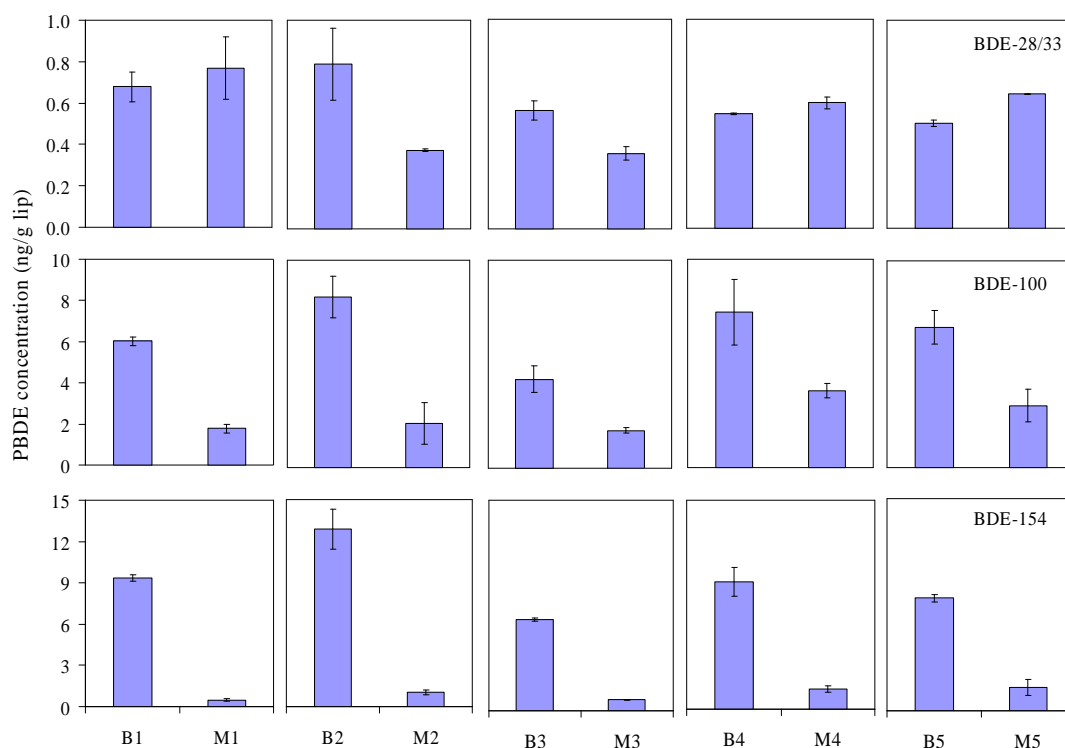


Figure 1. Mean ( $\pm$  range) concentrations (ng/g lipid) and range of selected PBDEs in blubber (B) and milk (M) of the five lactating females.

## Results and Discussion

Ten of the 39 PBDE congeners examined, namely BDE-28/33, -47, -49, -66, -99, -100, -153, -154 and -155, were found in the blubber and milk samples. BDE-183 was also found in most of these samples, but results appeared erratic and were not considered.

Mean concentrations (ng/g lipid) and range of selected PBDEs in lactating female blubber (B) and milk (M) are reported in Figure 1. The range of PBDE concentrations represents the first and the second sampling of each lactating female (Table 1). There was no systematic decrease or increase of PBDE concentrations in milk samples between the two samplings. Concentrations of PBDEs in both blubber and milk of each lactating female were relatively similar for low brominated compounds such as BDE-28/33 (3 bromine atoms). In contrast, higher brominated congeners, such as BDE-100 (5 bromine atoms) or BDE-154 (six bromines atoms) were clearly more concentrated in maternal blubber than in milk (Figure 1).

The efficiency of the lactational transfer of PBDEs appeared to be directly related to physico-chemical properties of these compounds as shown by the strong relationship observed between the milk: female blubber lipid normalized concentration ratio (MFBR) and log of octanol-water partitioning coefficient,  $K_{ow}$  (Figure 2). BDE-28/33 (log  $K_{ow}$  = 5.94) exhibited an average MFBR close to unity, indicative of a very efficient transfer for those low brominated congeners from the female blubber to the maternal milk. On the other hand, higher brominated PBDEs, such as BDE-153, -154 and -155 (log  $K_{ow}$  = 7.82 – 7.90), showed an average MFBR below 0.2 indicating that concentrations of those PBDEs in milk are about 5 times lower than in female blubber.

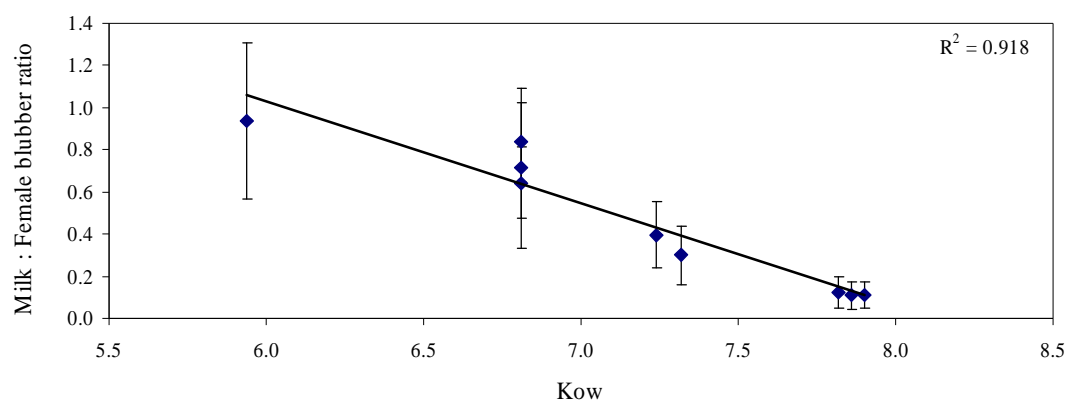


Figure 2. Mean ( $\pm$  std dev.) milk:female blubber ratios, MFBR, of PBDE congeners as a function of their log  $K_{ow}$ .  $K_{ow}$  values were taken from Braekevelt et al. 2003.

Despite the very brief lactation period and the massive transfer of fat exhibited by hooded seals, the lactational transfer of PBDEs from mother to pup is very selective, favouring the less brominated congeners. As a result, concentrations of PBDEs in milk, expressed on a lipid weight basis, strongly underestimate concentrations of high brominated PBDEs in blubber of the lactating females. Therefore, milk would not be a suitable tissue to represent females' contamination.

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